Human Cancer Syndromes: Clues to the Origin and Nature of Cancer

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More than 20 different hereditary cancer syndromes have now been defined and attributed to specific germline mutations in various inherited cancer genes. Collectively, the syndromes affect about 1 percent of cancer patients. An individual who carries a mutant allele of an inherited cancer gene has a variable risk of cancer that is influenced by the particular mutation, other cellular genes, and dietary, lifestyle, and environmental factors. Though hereditary cancer syndromes are rare, their study has provided powerful insights into more common forms of cancer. Somatic mutations in sporadic cancers frequently alter the inherited cancer genes, and the functions of cell signaling pathways have been illuminated by study of the affected genes. Further investigation of inherited mutations that affect susceptibility to cancer will aid efforts to effectively prevent, detect, and treat the disease.

Cancer is a genetic disease, arising from an accumulation of mutations that promote clonal selection of cells with increasingly aggressive behavior. The vast majority of mutations in cancer are somatic and are found only in an individual's cancer cells. However, about 1% of all cancers arise in individuals with an unmistakable hereditary cancer syndrome. These individuals carry a particular germline mutation in every cell of their body. Although rare, the inherited cancer syndromes are of vast biological importance. Studies of the specific mutations responsible for these syndromes and the cellular signaling pathways disrupted by the mutant proteins have begun to provide unprecedented insights into the molecular origins and pathogenesis of inherited and sporadic forms of cancer. I discuss (i) the strategies that have led to successful isolation of inherited cancer genes; (ii) the cellular signaling pathways that are disrupted by the mutant genes; (iii) the roles of allelic variation and modifier genes in cancer development; and (iv) some of the future challenges and opportunities for the field of cancer genetics.

Clues to Heritable Forms of Cancer

Family history has long been recognized as an important component of cancer risk, yet the identification of specific genes that affect cancer risk is a formidable task. Of critical importance in the discovery process has been the establishment of clear criteria for recognizing families and individuals who are not only likely to be affected by an inherited cancer syndrome, but who are also suitable for genetic studies. For instance, genetic studies have a greater likelihood of success in families in which multiple affected and unaffected individuals in two or more generations are available for analysis, than in families in which only a few individuals can be studied. A complicating factor in genetic studies is that cancer is not a single disease, even when it arises in the same organ site. Rather, it is a collection of many diseases, some of which are very common and others extremely rare. Thus, families in which multiple members develop a rare form of cancer, such as retinoblastoma or osteosarcoma, are much more likely to be segregating a mutation in an inherited cancer gene than are families affected by more common cancers, such as adenocarcinomas of the lung, breast, prostate, and colon. Nonetheless, an inherited cancer syndrome should be considered when numerous family members develop cancer at an especially young age or affected individuals develop multiple primary cancers, even if they are common cancers. Families in which those with cancer also manifest other rare conditions, particularly congenital abnormalities, should also arouse suspicion of a cancer syndrome.

However, in many families segregating a mutant copy (also known as a mutant "allele") of a major inherited cancer gene, none of these striking features will be evident, perhaps because of small family size, uncertain family history, or the absence of cancer in family members who carry the mutant allele (termed "incomplete penetrance"). Confounding matters further, in some families with an inherited cancer syndrome, sporadic cancers of the same type may arise in individuals who do not carry the mutant allele (termed "phenocopies"). Incomplete penetrance and phenocopies can make it difficult to distinguish true mendelian forms of cancer from chance familial aggregations. The number of mendelian forms of cancer is not known, but more than 20 distinct inherited syndromes have been defined (Table 1).

The term "inherited cancer genes" will be used here to describe those genes for which certain mutant alleles have been demonstrated to cause highly penetrant cancer syndromes when transmitted through the germline. As discussed below, the likelihood that an individual who carries a mutant allele of an inherited cancer gene will ultimately develop cancer is variable and dependent on the particular mutant allele; various other cellular genes that can influence the likelihood, age of onset, and severity of cancer (called modifier genes); and poorly understood dietary, lifestyle, and environmental factors. Hence, because variant alleles of modifier and other genes have a meaningful role in cancer development, the inherited cancer genes constitute only a subset of a larger class of genes that affect the cancer risk of an individual. This larger, more inclusive class of genes might be termed cancer susceptibility genes. Certain variant alleles of cancer susceptibility genes would be, by definition, associated with increased cancer risk. Either singly or collectively, these variant alleles may have an important role in sporadic cancers and familial aggregations of cancer that do not present as highly penetrant syndromes.

Mapping Inherited Cancer Genes

Linkage analysis remains the mainstay of efforts to map inherited cancer genes. This approach usually requires study of large, multigenerational families to establish that genetic markers from a particular chromosomal region cosegregate in unambiguous fashion with the development of cancer. Although linkage analyses have proven quite successful, they are sometimes limited by problems of variable penetrance and phenocopies, as noted above. Another obstacle is genetic heterogeneity, which refers to the fact that germline mutations in several different inherited cancer genes at unique chromosomal locations can give rise to essentially indistinguishable clinical syn-

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dromes. Genetic heterogeneity has been demonstrated or is suspected in inherited forms of melanoma, as well as colorectal, breast, and prostate cancer (Table 1).

The search for an inherited cancer gene can sometimes be greatly assisted by cytogenetic clues. Although gross chromosomal defects are rarely detected in the constitutional (normal) cells of patients with cancer, when observed, these alterations have helped to pinpoint the location of various inherited cancer genes. For example, in about 5% of retinoblastoma patients, interstitial deletions involving the retinoblastoma (*RB1*) and other genes in chromosome band 13q14 are seen (*1*). In a subset of patients with Wilms tumor and other rare conditions, interstitial deletions involving chromosome band 11p13 and the Wilms tumor (*WT1*) gene are observed (*2*). The observation of an interstitial deletion of chromosome band 5q21 in a patient with hundreds of intestinal polyps and no family history of cancer indicated that the adenomatous polyposis coli (*APC*) gene might be localized there (*3*). Similarly, balanced translocations involving chromosome 17q11 have been observed in constitutional cells of patients with neurofibromatosis type 1 (NF1) (*4*). To corroborate the cytogenetic findings, linkage analysis must be carried out on additional families with the particular cancer syndrome.

Traditional linkage approaches are most successful for mapping rare alleles that cause highly penetrant mendelian cancer syndromes. In reality, families with an obvious cancer syndrome are likely to represent only a small fraction of individuals with inherited predisposition to cancer. It is often estimated that upward of 10 to 15% of all cancers have a major inherited component, albeit one that may be enigmatic. Thus, mapping of the larger class of cancer susceptibility genes may rely increasingly on strategies other than linkage approaches, such as analysis of the segregation patterns of particular gene sequences in sibling pairs with cancer. Moreover, mapping strategies may ultimately be superseded. For instance, searches for statistically significant associations between variant alleles of putative

Table 1. Summary of selected inherited cancer syndromes. CHRPE, congenital hypertrophy of the retinal pigment epithelium; AML, acute myelogenous leukemia; GAP, GTPase-activating protein, a negative regulator of the p21 ras guanine nucleotide-binding proteins; contiguous gene disorder, alterations in several distinct genes in a particular chromosomal region account for the phenotype seen in patients with the disorder; hedgehog, a secreted factor that regulates cell fate determination via its binding to the PTCH protein; HGF, hepatocyte growth factor; GDNF, glial-derived neurotrophic factor.

Syndrome	Primary tumor	Associated cancers or traits	Chromosome location	Cloned gene	Proposed function of gene product
		Dominant inheritance			
Familial retinoblastoma	Retinoblastoma	Osteosarcoma	13q14.3	*RB1*	Cell cycle and transcriptional regulation; E2F binding
Li-Fraumeni Syndrome (LFS)	Sarcomas, breast cancer	Brain tumors, leukemia,	17p13.1	*p53 (TP53)*	Transcription factor; response to DNA damage and stress; apoptosis
Familial adenomatous polyposis (FAP)	Colorectal cancer	Colorectal adenomas, duodenal and gastric tumors, CHRPE jaw osteomas and desmoid tumors (Gardner syndrome) medulloblastoma (Turcot syndrome)	5q21	*APC*	Regulation of β-catenin; microtubule binding
Hereditary nonpolyposis colorectal cancer (HNPCC)	Colorectal cancer	Endometrial, ovarian, hepatobiliary and urinary tract cancer glioblastoma (Turcot syndrome)	2p16, 3p21 2q32, 7p22	*MSH2, MLH1 PMS1, PMS2*	DNA mismatch repair
Neurofibromatosis type 1 (NF1)	Neurofibromas	Neurofibrosarcoma, AML, brain tumors	17q11.2	*NF1*	GAP for p21 ras proteins; microtubule binding?
Neurofibromatosis type 2 (NF2)	Acoustic neuromas, meningiomas	Gliomas, ependymomas	22q12.2	*NF2*	Links membrane proteins to cytoskeleton?
Wilms tumor	Wilms tumor	WAGR (Wilms, aniridia, genitourinary abnormalities, mental retardation)	11p13	*WT1*	Transcriptional repressor
Wiedmann-Beckwith syndrome (WBS)	Wilms tumor	Organomegaly, hemi-hypertrophy hepatoblastoma, adrenocortical cancer	11p15	*?p57/KIP2 ?Others—contiguous gene disorder*	Cell cycle regulator
Nevoid basal cell carcinoma syndrome (NBCCS)	Basal cell skin cancer	Jaw cysts, palmar and plantar pits, medulloblastomas, ovarian fibromas	9q22.3	*PTCH*	Transmembrane receptor for hedgehog signaling molecule
Familial breast cancer 1	Breast cancer	Ovarian cancer	17q21	*BRCA1*	Interacts with Rad51 protein; repair of double-strand breaks
Familial breast cancer 2	Breast cancer	Male breast cancer, pancreatic cancer, ?others (for example, ovarian)	13q12	*BRCA2*	Interacts with Rad51 protein; ?repair of double-strand breaks
von Hippel-Lindau (VHL) syndrome	Renal cancer (clear cell)	Pheochromocytomas, retinal angiomas, hemangioblastomas	3p25	*VHL*	?Regulates transcriptional elongation by RNA polymerase II

cancer susceptibility genes and the development of cancer may prove valuable for identifying mutant alleles that have more subtle effects on cancer risk.

Identifying Inherited Cancer Genes

Once an inherited cancer gene has been mapped, candidate genes from the region can be isolated by positional cloning strategies or by database searches. Sequence-based analysis of the candidate genes remains the gold standard for identifying mutant alleles. Ultimately, to establish the authenticity of the candidate gene, the mutant alleles must be shown not only to segregate with cancer predisposition but to be causally involved in cancer development through functional studies. The simplest and most reliable classification scheme at present is that variant alleles with gain-of-function (activating) mutations in cancer are named oncogenes, and those genes in which both alleles have loss-of-function (inactivating) mutations in cancer cells are called tumor suppressor genes (5).

In addition to positional cloning strategies, three other investigative approaches have been applied successfully to the search for inherited cancer genes. Two of these rely on the fact that inherited cancer genes are often more frequently altered by somatic mutations than by germline mutations. The third approach relies on the hypothesis that mutations in cancer cells target a finite number of critical cellular pathways, and a mutation in any of several genes within a given pathway may have similar phenotypic consequences.

The first approach uses information gleaned from the chromosome deletions that arise in cancer cells, often referred to as allelic loss or loss of heterozygosity (LOH). Consistent with Knudson's "two-hit" hypothesis (6), the chromosome regions affected by LOH often harbor a tumor suppressor gene (7). In those individuals with a germline, inactivating mutation in a tumor suppressor allele, inactivation of the remaining allele often occurs in the cancer via LOH. In sporadic cancers, inactivation of both tumor suppressor alleles occurs somatically, and LOH is presumed to provide a growth advantage to the tumor cell because a prior somatic mutation has already

Table 1. (continued).

Syndrome	Primary tumor	Associated cancers or traits	Chromosome location	Cloned gene	Proposed function of gene product
Hereditary papillary renal cancer (HPRC)	Renal cancer (papillary type)	?Other cancers	7q31	*MET*	Transmembrane receptor for HGF
Familial melanoma	Melanoma	Pancreatic cancer, dysplastic nevi, atypical moles	9p21	*p16 (CDKN2)*	Inhibitor of CDK4 and CDK6 cyclin-dependent kinases
			12q13 ?Others	*CDK4*	Cyclin-dependent kinase
Multiple endocrine neoplasia type 1 (MEN1)	Pancreatic islet cell	Parathyroid hyperplasia, pituitary adenomas	11q13	*MEN1*	Unknown
Multiple endocrine neoplasia type 2 (MEN2)	Medullary thyroid cancer	Type 2A pheochromocytoma parathyroid hyperplasia Type 2B pheochromocytoma mucosal hamartoma Familial medullary thyroid cancer	10q11.2	*RET*	Transmembrane receptor tyrosine kinase for GDNF
Multiple exostoses	Exostoses (cartilaginous protuberances on bones)	Chondrosarcoma	8q24.1, 11p11-13 19p	*EXT1, EXT2* *EXT3*	Unknown Unknown
Cowden disease	Breast cancer, thyroid cancer (follicular type)	Intestinal hamartomous polyps, skin lesions	10q23	*PTEN (MMAC1)*	Dual-specificity phosphatase with similarity to tensin
Hereditary prostate cancer (HPC)	Prostate cancer	Unknown	1q25 ?Others	Unknown	Unknown
Palmoplantar keratoderma	Esophageal cancer	Leukoplakia	17q25	Unknown	Unknown
		Recessive syndromes			
Ataxia telangiectasia (AT)	Lymphoma	Cerebellar ataxia, immunodeficiency, ?breast cancer in heterozygotes	11q22	*ATM*	DNA repair; ?Induction of p53
Bloom's syndrome	Solid tumors	Immunodeficiency, small stature	15q26.1	*BLM*	?DNA helicase
Xeroderma pigmentosum	Skin cancer	Pigmentation abnormalities hypogonadism	Multiple complentation groups	*XPB, XPD* *XPA*	DNA repair helicases, nucleotide excision repair
Fanconi's anemia	AML	Pancytopenia, skeletal abnormalities	9q22.3 16q24.3 ?two others	*FACC* *FACA*	?DNA repair ?DNA repair

inactivated one tumor suppressor allele. LOH studies have aided in the localization of various inherited cancer genes, including those for neurofibromatosis type 2 (NF2), multiple endocrine neoplasia type 1 (MEN1), and the nevoid basal cell carcinoma syndrome (NBCCS) (*8–10*). In a fraction of cancers, deletions involve overlapping regions of both chromosomal homologs. These "homozygous deletions" are also presumed to inactivate a tumor suppressor gene in or near the deleted region. A powerful strategy, termed representational difference analysis (RDA), uses a clever combination of subtraction hybridization and cloning methods to isolate the specific DNA sequences affected by such deletions (*11*). To date, the RDA technology has aided in the cloning of two inherited cancer genes, namely, the *BRCA2* breast cancer susceptibility gene at chromosome 13q12 and the *PTEN/MMAC1* (Cowden disease) gene at 10q22-23 (Table 1) (*12, 13*).

The second approach, perhaps best termed the "positional candidate gene" strategy, has been crucial to the identification of other inherited cancer genes, including those responsible for multiple endocrine neoplasia type 2 (MEN2) and hereditary papillary renal cancer (HPRC) (*14, 15*). This approach depends on linkage analysis for gene mapping and subsequent sequence analysis for detection of germline mutations in known oncogenes or tumor suppressor genes in the implicated chromosomal region. For example, genome-wide linkage analyses were used to map MEN2 to chromosome 10q11-12 and HPRC to 7q31-34. Because LOH studies of the cancers arising in individuals with MEN2 or HPRC showed no LOH at chromosomes 10q and 7q, respectively, tumor suppressor gene defects were thought to be an unlikely basis for the syndromes. Previous studies had identified oncogenes in each of the regions linked to the syndromes, namely, *RET* at 10q11.2 and *MET* at 7q31. Sequence analysis revealed activating mutations in *RET* in patients with MEN2 and the related syndrome of familial medullary thyroid cancer (FMTC) (*14*), and activating mutations in *MET* in patients with HPRC (*15*).

The third approach exploits the increasing knowledge of genetic and biochemical pathways in normal and cancer cells. This "functional" approach has yielded critical insights into several cancer syndromes, including inherited melanoma and colorectal cancer. Linkage studies have localized at least one melanoma predisposition gene to chromosome 9p, and germline, inactivating mutations in the *p16/INK4a/MTS1/CDKN2* (hereafter referred to as *p16*) tumor suppressor gene on 9p have been identified in some families (*16, 17*). The p16 protein regulates the activity of the CDK4 cyclin-dependent cell cycle kinase (*18*). Families with inherited melanoma, but without germline p16 mutations, were examined for mutations in *CDK4*. Germline mutation in *CDK4* was found to underlie the predisposition to melanoma in two unrelated families (*19*). The mutant CDK4 protein is oncogenic, because it is insensitive to the inhibitory action of the p16 protein.

Another remarkable example of the functional approach is the case of hereditary nonpolyposis colorectal cancer (HNPCC) and the insights provided by prior studies of bacterial and yeast DNA repair pathway mutants. The presence of thousands of somatic mutations in short, repeated DNA sequence tracts in cancers from HNPCC patients, as well as some sporadic cancers, suggested that the cancer cells might have defects in DNA synthesis or repair [reviewed in (*20*) and (*21*)]. Yeast and bacterial geneticists recognized that the cancer cell phenotype resembled that of bacterial and yeast strains with DNA mismatch repair (MMR) gene defects. Linkage studies mapped distinct HNPCC genes to chromosomes 2p and 3p, and sequencing studies of the MMR genes that had been mapped to these particular chromosomes were undertaken in HNPCC patients. Collectively, inactivating mutations in the MSH2 MMR gene on chromosome 2p and the *MLH1* MMR gene on chromosome 3p have been found in roughly 65% of individuals affected by HNPCC (Table 1) (*21*). Defects in other MMR genes account for some of the remaining HNPCC cases.

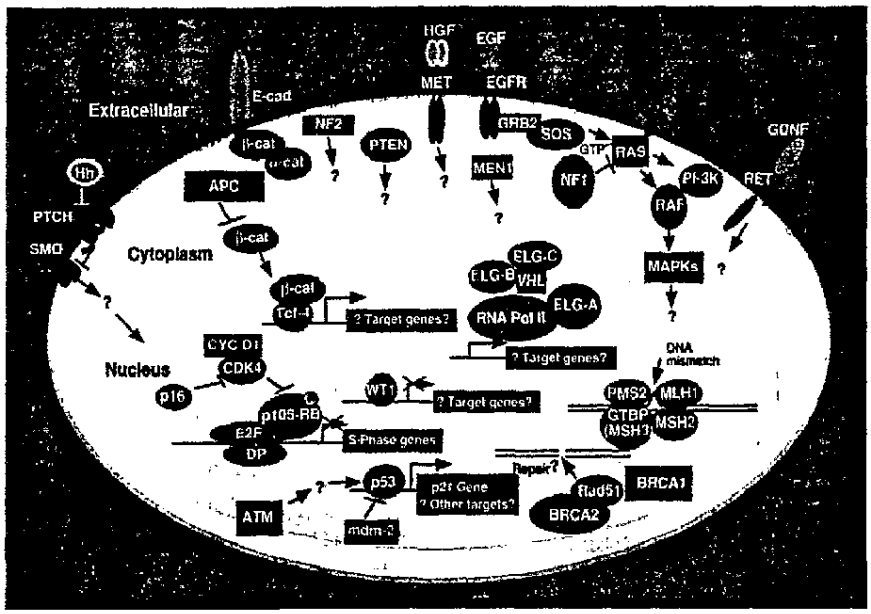

Fig. 1. Schematic representation of the cellular localization and presumed functions of the proteins encoded by inherited cancer genes (shown in magenta). Abbreviations: PTCH, patched; Hh, hedgehog; SMO, smoothened; HGF, hepatocyte growth factor; MET, HGF receptor; E-cad, E-cadherin; β-cat, β-catenin; α-cat, α-catenin; APC, adenomatous polyposis coli; Tcf-4, T cell factor 4; NF2, neurofibromatosis type 2 protein product; PTEN, phosphatase and tensin homolog deleted on chromosome 10 [also known as "mutated in multiple advanced cancers 1" (MMAC1)]; EGF, epidermal growth factor; EGFR, EGF receptor; GRB2, an adaptor protein with src homolog domains; SOS, a nucleotide exchange factor; PI-3K, phosphoinositide 3-OH kinase; RAS, p21 RAS guanine nucleotide-binding protein; RAF, serine/threonine kinase; NF1, neurofibromatosis type 1; MAPKs, mitogen-activated protein kinases; GDNF, glial-derived neutrophic factor; CYC D1, cyclin D1; CDK4, cyclin-dependent kinase 4; p105-RB, retinoblastoma protein; E2F, transcription factor family initially defined by their binding to the adenovirus E2 promoter; DP, family of E2F partner proteins needed for high-affinity binding to E2F sites; WT1, Wilms' tumor protein; VHL, von Hippel Lindau protein; ELG-A, -B, and -C, elongins A, B, and C; RNA Pol II, RNA polymerase II; MSH2, MLH1, PMS2, and GTBP3 (MSH3), DNA mismatch repair proteins; BRCA1 and BRCA2, proteins encoded by breast cancer susceptibility genes 1 and 2, respectively; ATM, ataxia telangiectasia, mutated; Rad51, human homolog of the yeast Rad51 DNA repair protein.

Functions of Inherited Cancer Genes

The proteins encoded by inherited cancer genes have been implicated in a diverse array of cellular processes, including proliferation, differentiation, apoptosis, and maintenance of genomic integrity. These proteins appear to function as transmembrane receptors (*MET, PTCH, RET*), cytoplasmic regulatory or structural proteins

(*NF1*, *PTEN APC*, *NF2*), transcription factors or regulators of transcription (*p53*, *WT1*, *RB1*, *VHL*), cell cycle factors (*CDK4* and *p16*), or DNA damage repair pathway proteins (*MSH2*, *MLH1*, *PMS2*, *ATM*, *BRCA1*, *BRCA2*, *FACC*, *FACA*, *XPA*, *XPB*, *XPD*, *BLM*) (Table 1 and Fig. 1).

Most of the inherited cancer syndromes show a dominant pattern of inheritance, and inactivating mutations in tumor suppressor genes, rather than activating mutations in oncogenes, predominate (Table 1). Consistent with Knudson's "two-hit" hypothesis, the germline tumor suppressor gene defect is recessive at the somatic cell level, and the corresponding normal allele is inactivated by somatic mutation during cancer development. Only three syndromes have been attributed to germline mutations in oncogenes (*RET*, *MET*, *CDK4*). In contrast to mutant tumor suppressor genes, activated oncogenes function dominantly in the cell, and somatic mutation of the other allele is not necessary for cancer development. Nevertheless, regardless of whether the germline mutation is in a tumor suppressor gene or an oncogene, additional somatic mutations are needed for cancer development.

Several rare recessive cancer syndromes, including ataxia telangiectasia (AT), Bloom's syndrome, xeroderma pigmentosum, and Fanconi's anemia, have been well described (Table 1). A common theme is that these syndromes result from germline inactivation of genes encoding DNA damage repair proteins. However, the specific cancer types and DNA-damaging agents that increase cancer risk are distinct in each syndrome. Whereas AT heterozygotes may have an increased risk of breast cancer (*22*), only homozygotes have a clearly increased cancer risk in other recessive cancer syndromes. These observations contrast with the picture in the dominant syndromes, where heterozygotes have elevated cancer risk. As noted above, the basis for increased cancer risk in an individual with a dominant cancer syndrome that arises from a germline tumor suppressor mutation is that the normal copy of the gene is often inactivated by a second "hit" in somatic cells. Thus, the observations imply that second "hits" in tumor suppressor genes of the type that underlie dominant cancer syndromes must have more potent effects on cancer initiation than second "hits" in tumor suppressor genes of the type that underlie recessive cancer syndromes.

In several syndromes for which genetic heterogeneity has been found—such as HNPCC, inherited melanoma, and breast cancer—all of the implicated genes appear to function in a conserved pathway (Fig. 1). Inactivation of *MSH2*, *MLH1*, and *PMS2* in patients with HNPCC alters the fidelity of DNA mismatch recognition and repair. Mutations in *p16* and *CDK4* in individuals with inherited predisposition to melanoma presumably alter cell cycle control, including phosphorylation of p105-RB and entry into the DNA synthesis (S) phase of the cell cycle. Recent studies of the BRCA1 and BRCA2 proteins suggest that both interact directly or indirectly with homologs of the yeast Rad51 DNA protein, which functions in the repair of double-stranded DNA breaks (*23*). Careful evaluation of other genes that function in a pathway already implicated in cancer syndromes would seem to have considerable merit as an approach to identifying novel mutations that underlie inherited cancer susceptibility.

Insights into Sporadic Cancers and Signaling Pathways

Investigations of inherited cancer genes have contributed substantially to our understanding of the somatic mutations present in sporadic cancers, as well as the function of cell signaling pathways. Studies of the *APC* gene illustrate these points well. Germline, inactivating mutations in APC are responsible for familial adenomatous polyposis, a rare condition affecting about 1 in 7000 individuals in the United States (*21*). Although germline mutations in APC are infrequent, somatic mutations in the APC gene are present in more than 70% of all adenomatous polyps and carcinomas of the colon and rectum (*21*). The APC protein of roughly 300 kD interacts with several cellular proteins, including β-catenin, an abundant protein initially identified because it bound to the cytoplasmic domain of the E-cadherin cell-cell adhesion molecule (*24*). APC not only interacts with β-catenin via two distinct sets of binding sites, but it can regulate β-catenin levels (Fig. 2) (*25*). The ability of APC to regulate β-catenin appears to depend on glycogen synthase kinase 3 (GSK3), a component of the WNT signaling pathway. Present data suggest that in the absence of WNT signaling, APC and GSK3 cooperate to degrade β-catenin (*26*). In response to WNT signaling via the Frizzled receptor, GSK3 is inhibited and β-catenin is stabilized [reviewed in (*27*)].

Inactivation of APC in colorectal cancers allows β-catenin to accumulate and complex with the Tcf-4 transcription factor, thereby activating the expression of

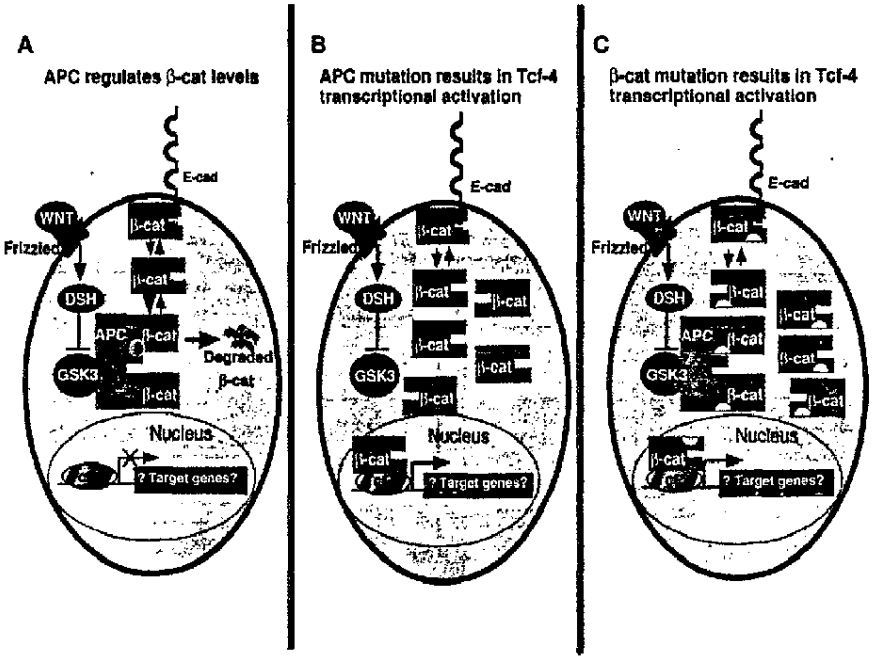

Fig. 2. APC protein regulates β-catenin (β-cat) levels in normal cells, and mutations in APC or β-cat in cancer cells deregulate cell growth via T cell factor 4 (Tcf-4) transcriptional activation. β-catenin is an abundant cellular protein, and much of it is often bound to the cytoplasmic domain of the E-cadherin (E-cad) cell-cell adhesion protein. (**A**) In normal cells, glycogen synthase kinase 3 (GSK3) and APC promote degradation of free β-cat, probably as a result of phosphorylation of the NH_2-terminal sequences of β-cat. GSK3 activity and β-cat degradation are inhibited by activation of the Wingless (WNT) pathway, as a result of the action of the Frizzled receptor and Dishevelled (DSH) signaling protein. (**B**) Mutation of APC in colorectal and other cancer cells results in accumulation of β-cat, binding to Tcf-4, and transcriptional activation of Tcf-4 target genes. (**C**) Point mutations and small deletions in β-cat in cancer cells inhibit phosphorylation and degradation of β-cat by GSK3 and APC, with resultant activation of Tcf-4 target genes.

Tcf-4–regulated genes (Fig. 2B) (28). Additional compelling evidence that gene activation by the β-catenin–Tcf-4 complex is a critical event in cancer development has been obtained. In a subset of the colorectal cancers that lack somatic mutations in APC, somatic mutations in β-catenin have been found. These mutations are presumed to render β-catenin insensitive to regulation by APC and GSK3. Consequently, β-catenin accumulates and activates Tcf-4–regulated genes (Fig. 2C) (29). Somatic mutations in β-catenin or APC are also present in some melanomas (30). Further work is now needed to identify the specific genes regulated by Tcf-4 and their role in cancer development.

Tissue Specificity of Gene Defects

Despite the considerable insights into inherited cancer gene function, many puzzling observations remain. The vast majority of inherited cancer genes appear to be expressed in most adult tissues, yet individuals carrying a germline mutation in these genes often manifest only a limited spectrum of cancers. For instance, *RB1* germline mutations predispose to retinoblastoma and osteosarcoma, and rarely to soft tissue sarcoma and melanoma (*31*, *32*). Germline *p53* mutations predispose primarily to osteosarcoma, soft tissue sarcoma, brain tumors, leukemia, and breast cancer in women (*31*, *33*), whereas *p16* germline mutations predispose to melanoma and pancreatic cancer (*16*, *17*). These observations are curious, because somatic mutations in *RB1*, *p16*, or *p53* are believed to be causally involved in a substantial fraction of many different sporadic cancers, including lung and colon cancers. Notably, these and many other common cancers do not arise at increased frequency in patients with germline *RB1*, *p53*, or *p16* mutations.

Although genetic and biochemical data support the proposed protein functions and interactions depicted in Fig. 1, the situation in vivo is undoubtedly far more complex. For example, Fig. 1 suggests that the phenotypic consequences of *p16* or *RB1* inactivation are likely to be equivalent; however, as noted above, patients with germline mutations in the *RB1* and *p16* genes are predisposed to distinct cancer types. Similarly, despite early indications that *BRCA1* and *BRCA2* function in the same cellular processes, mutations in the two genes are associated with different cancer profiles. Women with germline *BRCA1* mutations exhibit principally breast and ovarian carcinoma (*34*), whereas germline *BRCA2* mutations predispose to female and male breast carcinoma as well as other cancer types, such as pancreatic adenocarcinoma (*35*). As yet, there is no compelling explanation for these observations. Certain inherited cancer genes, such as *p16*, *CDK4*, and *RB1*, are likely to function in intersecting or overlapping genetic pathways, but these pathways may branch considerably or the genes may have entirely distinct functions, perhaps depending on cell type.

Although the concepts of branching pathways or alternative gene function in different tissues may help to explain the tissue-specific cancer spectra in mutation carriers, in the case of HNPCC, this explanation seems dubious. Presumably, the mismatch repair genes have the same function in all cell types. Thus, other explanations must be considered. For instance, in an individual with HNPCC, inactivation of the remaining normal copy of the mismatch repair gene may occur at increased frequency in colonic epithelial cells, either as a result of endogenous cellular processes or specific environmental and dietary effects. The inactivating event could be a somatic mutation or even an epigenetic change, such as DNA methylation.

Allelic Variation and Modifier Genes

As noted above, germline mutations in the RET transmembrane tyrosine kinase receptor have been identified in patients with MEN2 and FMTC. Although all of these mutations appear to produce a gain-of-function defect (constitutive activation of the kinase), specific mutations are correlated with specific disease features (Fig. 3). Families with the MEN2A subtype of MEN2 develop medullary thyroid cancer (MTC), parathyroid hyperplasia, and pheochromocytoma, whereas families with MEN2B develop MTC and mucosal ganglioneuromas of the lips, tongue, and intestinal tract, but not parathyroid hyperplasia. In FMTC, only MTCs are found. Missense mutations in one of five cysteines of the RET extracellular domain are present in nearly all cases of MEN2A and FMTC, and presumably constitutively activate RET's tyrosine kinase activity by mimicking the effects of ligand binding to the extracellular domain (Fig. 3) (*14*, *36*). Almost all MEN2B pa-

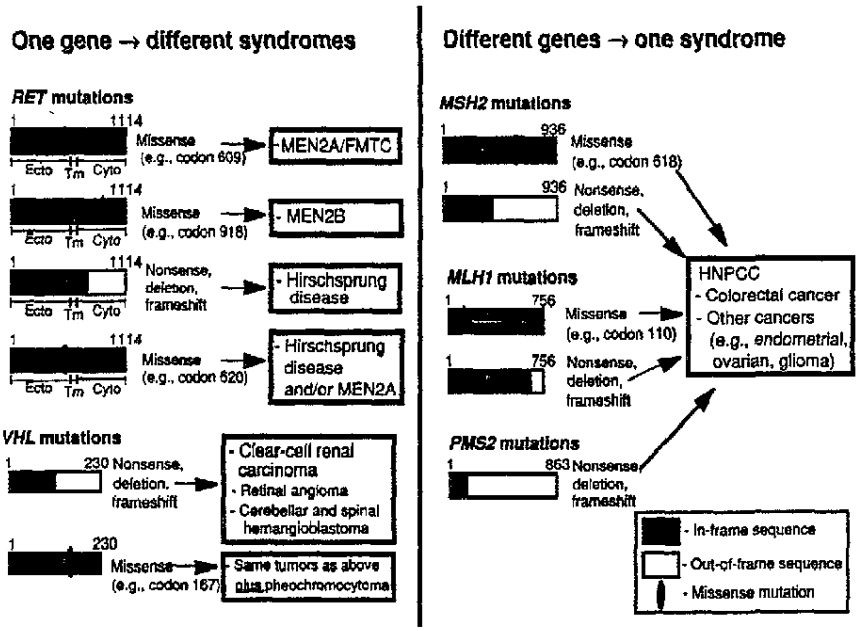

Fig. 3. *The roles of allelic variation (left panel) and genetic heterogeneity (right panel) in inherited cancer* syndromes are illustrated. In individuals with MEN2A or FMTC, missense mutations in one of five cysteines in the extracellular domain of the RET transmembrane receptor tyrosine kinase mimic the effects of ligand binding. As a result, the receptor is constitutively activated, causing medullary thyroid cancer and other features of MEN2A and FMTC. In those with the MEN2B variant, mutation of a critical amino acid in the kinase domain activates the receptor and alters its substrate specificity. Inactivating mutations in *RET* lead to the colonic features seen in Hirschsprung disease. In some families, mutations in RET extracellular cysteines can also cause Hirschsprung disease, with or without the features of MEN2A. Depending on the specific VHL mutation, variable disease features are seen. Inactivating mutations (such as nonsense mutations or deletions) predispose to clear-cell renal carcinoma, retinal angioma, and cerebellar and spinal hemangioblastoma. Missense mutations predispose to these tumors, as well as pheochromocytoma. In patients with HNPCC, germline mutation of a mismatch repair gene, such as the MSH2, MLH1, or PMS2 gene, predisposes to colorectal and other cancers. No clear-cut differences in disease features are seen among mutation carriers, regardless of the specific gene affected or the type of mutation.

tients have a missense mutation at a single critical residue in the cytoplasmic tyrosine kinase domain of RET (*14*, *36*). This mutation presumably activates the kinase and alters its substrate specificity.

Thus, *RET* allelic variation appears to distinguish MEN2B from MEN2A and FMTC, but it does not account for the differences between MEN2A and FMTC, and modifier genes must be considered. Other data also support a role for modifier genes or other factors in the pathogenesis of MEN2 and FMTC. Missense mutations in RET extracellular domain cysteines have also been detected in patients with Hirschsprung disease, a congenital syndrome in which absence of parasympathetic intrinsic ganglion cells in the colon leads to recurrent severe constipation or intestinal obstruction, or both (*37*). Many families with autosomal dominant Hirschsprung disease have inactivating mutations in one *RET* allele (Fig. 3), suggesting that loss of RET function is responsible for the colonic features of the disease (*14*, *36*). Hence, it is surprising that, in some families, germline missense mutations in the RET extracellular domain cysteines cosegregate with MEN2A/FMTC and Hirschsprung disease features (*38*). One possible explanation is that particular mutant *RET* alleles may promote neoplastic transformation of certain cell types (for example, the so-called "C" cells of the thyroid) but induce apoptosis in others (for example, enteric ganglion cells). Alternatively, some mutant *RET* alleles may have gain of function in one cell type and loss of function in another.

Another cancer syndrome that illustrates the effects of allelic variation and modifier genes is familial adenomatous polyposis (FAP). In the majority of FAP patients, germline APC mutations result in COOH-terminally truncated proteins. Considerable variation can be seen among FAP patients, both in the spectrum of extracolonic features and in the severity of the intestinal polyposis phenotype. This phenotypic variation depends, in part, on the specific APC mutation [reviewed in (*21*)]. Patients with the Gardner syndrome variant of FAP have polyposis and extracolonic manifestations, including desmoid tumors and jaw osteomas; these patients typically have APC mutations between codons 1403 and 1578. Truncating mutations COOH-terminal to APC codon 1924 have also been associated with desmoid tumor development (*39*). Nonmalignant retinal lesions known as congenital hypertrophy of the retinal pigment epithelium (CHRPE) are often seen in patients with mutations between APC codons 463 and 1387. Truncating mutations before APC codon 160 or COOH-terminal to codon 1920 are associated with attenuated forms of polyposis (*21*). Clear molecular explanations for the relationships between particular APC mutations and the phenotypes seen in patients are lacking.

Interestingly, there may be considerable phenotypic differences among patients who inherit the same mutant APC allele. In fact, identical *APC* germline mutations have been found in three distinct groups of patients: those with only intestinal polyposis; those with Gardner syndrome; and those with polyposis and medulloblastoma (*21*). These findings suggest that modifier genes may have a significant effect on FAP phenotype, and studies of a mouse genetic model for FAP, known as Min (for multiple intestinal neoplasia), have further supported this view. The *Min* mutation, like those in many FAP patients, causes premature truncation of the APC protein, and mice heterozygous for the *Min* allele develop multiple adenomatous polyps in their intestine (*40*). Depending on the inbred strain carrying the *Min* allele, wide variations in polyp number are seen. Linkage analysis has demonstrated that much of the variation is due to a single locus, named *Mom-1* (modifier of *Min*), which encodes a secreted phospholipase A2 (*41*). Other studies of mouse FAP models have revealed that genes encoding DNA methyltransferase and cyclooxygenase 2 also have significant effects on the intestinal phenotype (*42*).

Studies of individuals with *VHL* or *BRCA1* mutations have also identified relationships between specific mutant alleles and the spectrum of disease features seen (Fig. 3) (*43*). However, in HNPCC patients, there appears to be little correlation between the particular mismatch repair gene defect or the type of mutation (missense, nonsense, frameshift) and the specific clinical phenotype seen (Fig. 3) (*21*). Individuals with germline mutations in DNA mismatch repair genes are at increased risk of colorectal and other cancers, including those of the endometrium, ovary, and brain. There is variability in phenotype among those who carry identical germline mutations, and this variability is likely due to other genetic and environmental influences.

Future Challenges and Opportunities

It was only about 10 years ago that the first inherited cancer gene, *RB1*, was cloned and sequenced. Now, there is data on more than 25 inherited cancer genes (Table 1). Despite this success, substantial challenges remain in the cancer genetics field.

Information is needed about the prevalence of germline mutations in inherited cancer genes in individuals with apparently sporadic forms of cancer and the population at large, if we wish to understand cancer risk in mutation carriers. Present technologies for identifying mutations are not sufficiently rapid, robust, or economical for tackling these questions. For example, familial retinoblastoma is presumed to be a genetically homogeneous disease, yet mutation detection rates for the *RB1* gene vary from 40 to 85% in different studies (*44*). Even for FAP, where APC mutations are detected in nearly 75% of patients, the detection strategies are costly and cumbersome. New "chip-based" DNA technologies may soon revolutionize analysis, but crucial issues remain unresolved, including the availability and cost of such approaches. In addition, functional assays may be needed to assess the significance of rare variant alleles, particularly those with missense substitutions or alterations in noncoding sequences.

Although associations have been noted for particular mutant alleles of inherited cancer genes and the age of onset, severity, and types of cancer that arise in gene carriers, it has become abundantly clear that other genes and dietary, environmental, and lifestyle factors substantially modify the expression of cancer in mutation carriers. Mouse models of inherited cancer have already proven valuable for identifying modifier genes, and the models are also likely to yield new insights into the means by which dietary and environmental agents affect cancer risk. Nonetheless, some cautionary flags have been raised. Several mouse models of inherited cancer, including mice with *Rb* and *Nf2* defects, do not develop the tumor types seen in humans, and, in fact, develop tumors not seen in humans with the corresponding germline defect (*45*). In addition, mice heterozygous for defects in homologs of the *BRCA1*, *BRCA2*, *WT1*, and *VHL* genes have not shown elevated rates of spontaneous cancer (*45*, *46*). These differences in cancer spectrum and incidence may be attributable to the specific genetic backgrounds of the few inbred mouse strains in which the mutations have been studied thus far. Alternatively, the differences may truly reflect species-specific differences.

An important but poorly understood area is the role of low-penetrance genes in cancer susceptibility. Cancers only rarely display clear-cut patterns of mendelian inheritance, yet many types show an increased propensity to arise in families. An intriguing notion is that some familial aggregations result from subtle or "unconventional" mutations in known inherited cancer genes, such as *APC*, *BRCA1*, and *MSH2*. Consistent with this proposal, recent studies indicate that a germline APC mutation that does not alter the function of

the encoded protein may be a major contributing factor in approximately 10% of colorectal cancers in Ashkenazi Jews (47). The mutation generates a hypermutable tract in the *APC* sequence, and somatic mutations are presumed to arise at increased frequency in or near the tract. Other familial aggregations may reflect interactions between mutant alleles of inherited cancer genes and modifier genes. In some families and individuals, cancer risk may be attributable to variant alleles of genes that regulate cell metabolism or the response to environmental and dietary agents and toxins (48).

Research into the genetics of inherited cancer syndromes has provided fundamental insights into the cellular defects that subvert normal cell growth and lead to the insidious and destructive properties of cancer. Further identification and study of genes that influence cancer susceptibility will likely provide an ever clearer understanding of the origin and nature of cancer, as well as form the foundation for efforts to effectively prevent, detect, and treat it.

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Genetic Testing for Cancer Risk

Bruce Ponder

Genetic testing for cancer susceptibility is already part of the clinical management of families with some of the well-defined (but uncommon) inherited cancer syndromes. In cases where the risks associated with a predisposing mutation are less certain, or where there is no clearly effective intervention to offer those with a positive result, its use is more controversial. Careful evaluation of costs and benefits, and of the efficacy of interventions in those found to be at risk, is essential and is only just beginning. An immediate challenge is to ensure that both health professionals and the public understand clearly the issues involved.

With the cloning of cancer-predisposing genes over the past 10 years, it has become possible to offer predictive DNA testing to family members at risk. This procedure has been quietly and successfully applied by specialist clinics to several inherited cancers—for example, retinoblastoma, polyposis coli, and multiple endocrine neoplasia type 2 (MEN-2) (*1*). With the cloning of the *BRCA1* and *BRCA2* genes that predispose to breast and ovarian cancer, however, a small storm has blown up (*2*–*7*). The acceptance of testing for other inherited cancers suggests that there is nothing intrinsically contentious about testing for cancer genes. For breast cancer, the most important difference is that it is not clear whether it is necessarily helpful for a patient to know that she has a *BRCA1* or *BRCA2* mutation. In addition, breast cancer affects a very large number of people, and it seems probable that neither the patients nor their doctors fully understand what is involved in deciding to take

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